Current Biology 24, 1369–1374, June 16, 2014 ©2014 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.04.053

Report

Rapid Convergent Evolution in Wild Crickets

Sonia Pascoal,[1] Timothee Cezard,[2] Aasta Eik-Nes,[1] Karim Gharbi,[2] Jagoda Majewska,[3] Elizabeth Payne,[4] Michael G. Ritchie,[1] Marlene Zuk,[4,5] and Nathan W. Bailey[1,*]

[1]Centre for Biological Diversity, University of St Andrews, St Andrews, Fife KY16 9TH, UK

[2]Edinburgh Genomics, University of Edinburgh, Edinburgh EH9 3JT, UK

[3]Institute of Environmental Sciences, Jagiellonian University, Gronostajova 7, 30-387 Kraków, Poland

[4]Department of Biology, University of California, Riverside, CA 92521, USA

[5]Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul, MN 55108, USA

Summary

The earliest stages of convergent evolution are difficult to observe in the wild, limiting our understanding of the incipient genomic architecture underlying convergent phenotypes [1, 2]. To address this, we capitalized on a novel trait, flatwing, that arose and proliferated at the start of the 21st century in a population of field crickets (*Teleogryllus oceanicus*) on the Hawaiian island of Kauai [3]. Flatwing erases sound-producing structures on male forewings. Mutant males cannot sing to attract females, but they are protected from fatal attack by an acoustically orienting parasitoid fly (*Ormia ochracea*). Two years later, the silent morph appeared on the neighboring island of Oahu. We tested two hypotheses for the evolutionary origin of flatwings in Hawaii: (1) that the silent morph originated on Kauai and subsequently introgressed into Oahu and (2) that flatwing originated independently on each island. Morphometric analysis of male wings revealed that Kauai flatwings almost completely lack typical derived structures, whereas Oahu flatwings retain noticeably more wild-type wing venation. Using standard genetic crosses, we confirmed that the mutation segregates as a single-locus, sex-linked Mendelian trait on both islands. However, genome-wide scans using RAD-seq recovered almost completely distinct markers linked with flatwing on each island. The patterns of allelic association with flatwing on either island reveal different genomic architectures consistent with the timing of two mutational events on the X chromosome. Divergent wing morphologies linked to different loci thus cause identical behavioral outcomes—silence—illustrating the power of selection to rapidly shape convergent adaptations from distinct genomic starting points.

Results and Discussion

When discrete populations adapt to similar selective regimes, convergent phenotypic outcomes often evolve. Whether such traits arise from different genotypic changes or shared ancestry is of great interest, because such information provides insight into both genetic constraints and common genetic pathways for adaptive evolution. The initial sequence changes that set lineages on divergent genetic but convergent phenotypic trajectories have until now been difficult to detect in wild systems, but different starting points can have critical impacts on subsequent genetic and phenotypic evolution [4]. These genetic underpinnings could be as varied as point mutations inducing single-amino-acid changes, different deletions in the same gene, similar but independent genetic changes, gene amplifications, or changes across vast suites of quantitative loci that produce similar adaptations [4–7]. Understanding the degree to which the initial genetic architecture of convergent phenotypes shapes how rapidly and consistently different populations or species evolve phenotypic solutions to the same selective pressures is a challenge [8]. Here we capitalize on a system of rapidly evolving wild field crickets (*Teleogryllus oceanicus*) to tackle that challenge.

The abrupt appearance and rapid proliferation of silent flatwing crickets on the Hawaiian island of Kauai is a textbook case of rapid evolution in the wild [9]. Remarkably, the initial discovery of flatwing on Kauai was followed by a seemingly identical effect in a separate population just 2 years later (Figure 1 and Table S1 available online) [3]. The evolutionary explanation for this pattern is unclear. Given the proximity of the two islands and the opportunity for interisland migration through agricultural and tourist transport, it seemed plausible that the mutation originated in Kauai and then migrated to Oahu, where it introgressed and spread under similar selection pressure. Comparable examples of rapid introgression have been observed in other organisms, including *Heliconius* butterflies and house mice (*Mus spp.*) [10–12]. Alternatively, a distinct mutation could have arisen independently on Oahu and evolved under pressure from the parasitoid. This situation provides a unique opportunity to investigate the genomic architecture of the earliest stages of adaptive convergent evolution in the wild. We evaluated these scenarios by performing a morphometric analysis of wings from Kauai and Oahu males, establishing the mode of inheritance of the mutation on Oahu to complement existing information about its mode of inheritance on Kauai, and performing genome-wide analyses to identify markers associated with flatwing in each population. The key test in the genomic analysis was whether flatwing-associated markers were the same or different in either population.

Silent Flatwings Differ Morphologically between Islands

Mutant flatwings represent a dramatic departure from normal male wings, as expected. A geometric morphometric analysis of flatwing and normal wing venation from males reared in a common-garden lab environment for at least two generations confirmed that the two male forms are distinct (Figure 2A) (multivariance analysis of variance [MANOVA] on principal components analysis [PCA] scores: $F_{84,858} = 39.89$, $p < 0.001$). A subsequent canonical variates analysis (CVA) illustrated the main differences (CVA: n = 315; CVA1: $\chi^2_{(84)} = 1{,}423.93$, eigenvalue = 41.99, $p < 0.001$) (Figure 2A). Surprisingly, flatwing venation was unambiguously distinct between Kauai and Oahu, with Oahu flatwings more closely resembling normal-wing males (Figure 2A). This was confirmed in

*Correspondence: nwb3@st-andrews.ac.uk

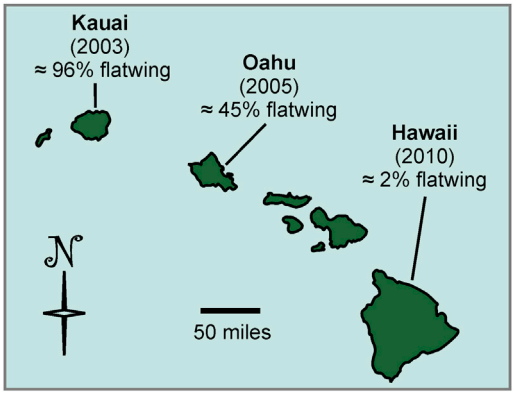

Figure 1. Sampled Populations

The map shows Hawaiian islands on which flatwing males are found and the year they were first documented. Although several flatwing males have recently been found on the "Big Island," Hawaii, the lab population derived from that island was established prior to 2012 and did not contain flatwing males at the time. See also Table S1.

analyses of the same landmark data that examined population-level differences in flatwings separately from population-level differences in normal males (MANOVA on flatwing PCA scores: $F_{28,169} = 26.41$, $p < 0.001$; MANOVA on normal PCA scores: $F_{28,88} = 3.12$, $p < 0.001$). CVAs confirmed these results and illustrate the key differences between groups (flatwing CVA1: n = 198, $\chi^2_{(28)} = 306.08$, eigenvalue = 4.38, $p < 0.001$; normal male CVA1: n = 117, $\chi^2_{(28)} = 69.67$, eigenvalue = 0.99, $p < 0.001$). The difference in eigenvalues for the latter two analyses indicated that the two samples of flatwings were approximately four times more different from one another than the two samples of normal wings were from each other (Figure 2B).

Differences between Kauai and Oahu flatwings are noticeable to the naked eye, with Kauai flatwings (hereafter designated fw^K) exhibiting a more drastic loss of secondary wing structures necessary for acoustic communication compared to Oahu flatwings (fw^O), which tend to retain a portion of their harp and cross veins (Figures 2C and 2D). Nevertheless, neither fw^K nor fw^O males can produce acoustic signals. Such a striking difference between mutants on the two islands was unexpected. To rule out the possibility that they were an artifact of sampling effects or laboratory conditions, we performed two independent replication studies. The first used separate lab-reared individuals from the same populations, which were sampled for the genomic analysis below, and the second used field-caught flatwing males. These yielded qualitatively identical results (Figure S1).

Flatwing Is Inherited as a Mendelian, Sex-Linked Mutation on Both Islands

Given the morphological differences between flatwing males from Kauai and Oahu, we next determined whether the mode of inheritance of the mutation was similar in both populations. Tinghitella [13] found that flatwing segregates in Mendelian fashion as a sex-linked, single-locus trait on Kauai, and we found that fw^O segregates in the same manner on Oahu. Field crickets have an XX/XO mode of sex determination, and a replicated series of crosses and backcrosses designed to test three modes of Mendelian inheritance—autosomal dominant, autosomal recessive, and sex linked—enabled us to rule out all but the latter for Oahu flatwings (Table 1). Females, not males, determine the phenotype of their male offspring, and the causal mutation segregates as a single-locus trait on the X chromosome in both populations.

Flatwing Cosegregates with Different Sets of Genomic Markers on Either Islan

Despite the striking variation in flatwing morphology between Kauai and Oahu, the shared mode of inheritance allowed for the possibility that a single mutation could produce different phenotypes on the islands due to genetic background effects. To test this in the absence of a reference genome, we applied bulk segregant analysis (BSA) using restriction-site-associated DNA sequencing (RAD-seq) [14] to identify single-nucleotide polymorphisms (SNPs) in linkage disequilibrium with the flatwing phenotype in each population. If the observed population-level morphological differences are caused by expression of the same sequence variant in different genomic backgrounds after introgression, then the majority of linked SNPs should be shared between the two populations. In contrast, if the two wing-silencing phenotypes are caused by sequence variants that affect genetically distinct regions of the X chromosome, then the BSA should recover nonoverlapping sets of linked SNPs for each island.

RAD analysis of DNA pooled from 100 lab-reared males of each morph in each population yielded 645,000 tags from which SNPs were detected (Figure S2). Because the mutation appears to have arisen so recently, allelic identity at loci associated with flatwing was expected to be fixed, owing to their physical linkage with the original causative mutation. Accordingly, we set the major allele frequency threshold for associated SNPs to 0.95 in flatwing pools and allowed allele frequencies to vary in normal pools. We called and filtered SNPs to ensure that each locus was represented with adequate sequencing coverage in each pool, enabling accurate comparison of SNP allele frequencies between populations (see the Supplemental Experimental Procedures). We identified 7,226 flatwing-associated SNP markers with almost no overlap between Kauai and Oahu (Figure 3A). Only 0.30% (n = 22) of all associated SNPs were shared between islands (Figure 3A). In addition, the number of associated markers for Oahu was an order of magnitude higher than for Kauai (n = 6,492 versus n = 756, respectively; Figure 3A), despite the power to detect SNPs being equivalent in both pools. This difference is consistent with flatwing arising on Oahu more recently and thus being flanked by a larger physical linkage block.

Allelic Identity at Loci Associated with Flatwing Differs between Islands

It was theoretically possible that the 22 shared SNPs are linked to the same causal mutation, which, when expressed in different backgrounds, produces different phenotypes. However, we believe that this is unlikely, due to the abundance of associated SNPs unique to each population, plus a previous *T. oceanicus* microsatellite study suggesting low genetic differentiation between populations on the two islands ($F_{ST} = 0.0369$) [15]. A final piece of evidence inconsistent with a scenario involving a single, introgressed mutation is that a surprising allelic reversal exists between populations. For a given SNP marker identified in the BSA, the major allele associated with Kauai flatwings tended to be the same as the major allele for Oahu normal males, and vice versa. Thus, at a given associated SNP marker, the alleles associated with flatwing are

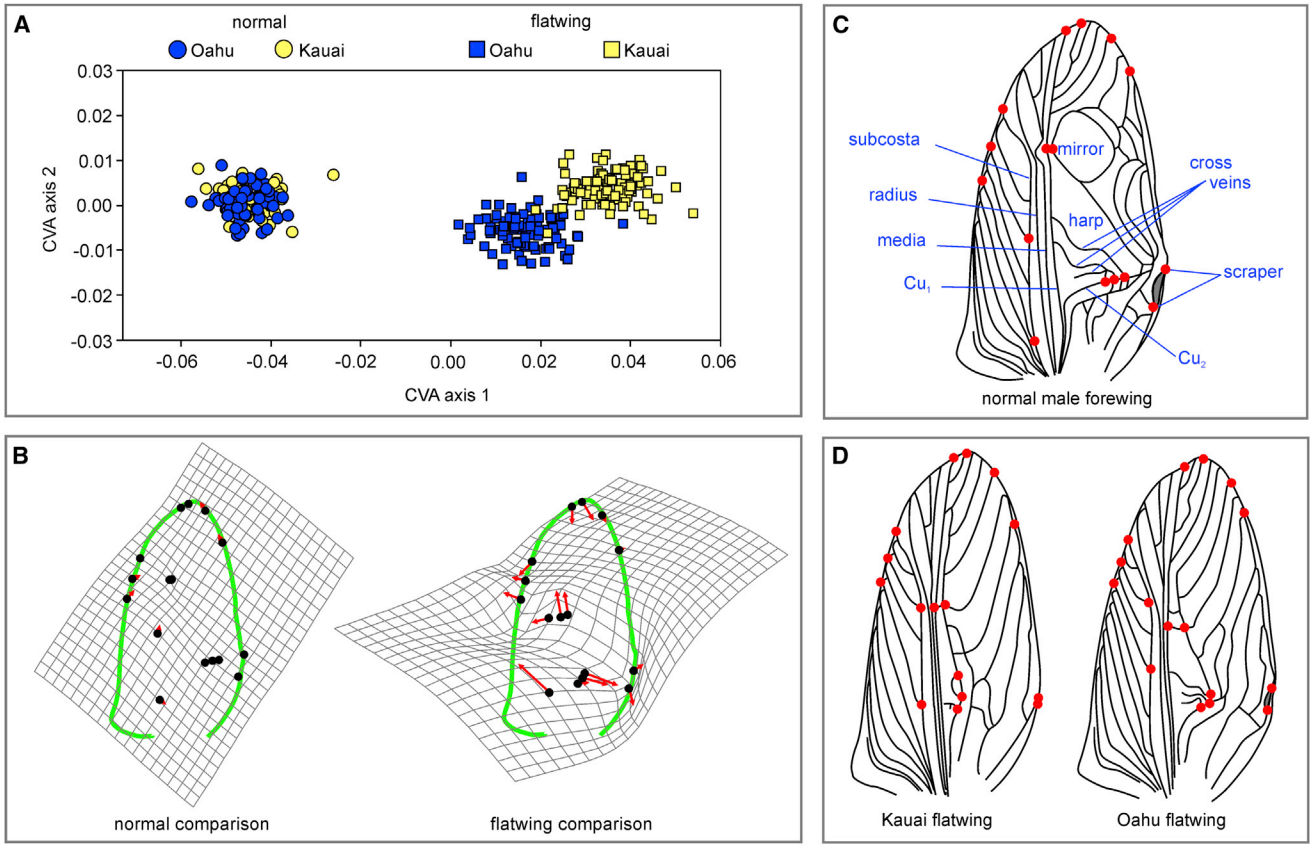

Figure 2. Morphological Differences between Male Wing Morphs and between Islands

(A) Canonical variate analysis (CVA) plot illustrating wing differences between morphs and populations along the first two significant CVA axes.

(B) Deformation grids illustrating areas of male wings that show divergence in shape between Kauai and Oahu for normal wings (left) and flatwings (right). Black dots indicate landmarks, and green outlines provide an approximate visual reference for landmark locations on the wing surface. The red arrows are vectors that describe the relative differences in landmark position between Kauai and Oahu. The figures are scaled so that the magnitude of differences between Kauai and Oahu flatwings can be compared to the magnitude of differences between Kauai and Oahu normal males.

(C) Typical normal male wing highlighting principal veins and oscillating structures for song production. The 16 homologous landmarks used in this study are indicated by red dots.

(D) Diagrams of randomly chosen Kauai and Oahu flatwings from this study, with red dots indicating landmark positions.

See also Figure S1 and Table S3.

reversed between the two populations. The overall allelic reversal across all associated SNPs was 70%. However, when we relaxed the major allele frequency threshold for designating a SNP as "associated," the proportion of loci showing allelic reversal dropped to approximately 10% (Figure 3B), indicating that the allelic reversal was a property of loci in linkage disequilibrium with the causative sequence variation underlying the flatwing phenotypes, and not a general phenomenon arising from variation between Kauai and Oahu genomes. Given the unexpected nature of the allelic reversal, we validated ten SNP markers using Sanger sequencing and excluded the possibility of sample mislabeling (see the Supplemental Experimental Procedures and Table S2). Thus, for the same loci, different alleles are associated with the flatwing mutation in the two populations. This is the expected pattern if mutations on different homologs of the X chromosome recently, and independently, arose in each population (Figures 3C and 3D).

Incipient Convergent Evolution

The fact that Kauai and Oahu flatwing forms evolved so recently opens a rare opportunity to study the initial genetic architecture underlying a convergent behavioral phenotype. Female-like wings have been documented in male mantids [16], and the presence of flatwing males in crosses where none were expected in both this study and in a previous one [13] (Table 1) suggests that male wing abnormalities might arise more frequently than expected due to mutations on the X chromosome. The X is more prone to accumulating male-antagonistic mutations in systems with female-homogametic sex determination, and the evolutionary loss or reduction of wings (brachyptery) is widespread and has independently arisen in numerous insect lineages [17]. In many instances, winglessness appears to involve a simple, single-locus genetic change that alters the hormonal control of wing production during development [18], but in other cases, brachyptery is environmentally induced and could thereby reveal cryptic genetic variation upon which selection can act [19]. The clear parallel with the loss of derived wing structures in *T. oceanicus* males bolsters the plausibility of multiple independent losses of song-producing structures. Convergent trait loss has been inferred in other systems—for example, loss of schooling behavior in the tetra *Astyanax mexicanus* [20]—and predation pressure has been identified

Table 1. Observed and Expected Frequencies of the Two Male Wing Morphs in Oahu Crosses, Assuming Different Modes of Inheritance

Cross	Observed Ratios (Normal:Flatwing)	Expected Ratios under Different Modes of Inheritance[a]		
		Autosomal Dominant	Autosomal Recessive	Sex Linked
F_1	960:1	0:1	1:0	1:0
F_2	372:445	1:3	3:1	1:1
		G_1 = 161.37, $p < 0.001$	G_1 = 321.77, $p < 0.001$	G_1 = 6.53, $p = 0.011$
BC1	264:301	0:1	1:1	1:1
			G_1 = 2.42, $p = 0.12$	G_1 = 2.42, $p = 0.12$
BC2	773:2	1:1	1:0	1:0
		G_1 = 1,046.54, $p < 0.001$		

[a]Goodness-of-fit test statistics are in parentheses. G tests that were significant after Bonferroni correction for multiple comparisons (n = 6, $\alpha = 0.008$) are indicated by underlined ratios. Visual inspection was performed for cases containing structural zeros, and deviation from the expected frequency was unambiguous. These cells therefore indicate male morph ratios that are incompatible with the indicated mode of inheritance and cross type.

as a potent driver of convergence [21]. The key selective agent underlying the rapid evolution and maintenance of divergent wing morphs in *T. oceanicus* is most likely the presence of the acoustically orienting parasitoid on both Kauai and Oahu, without which the phenotype would have no selective advantage [3].

Previous work on insecticide resistance has illuminated the genetic architecture and role of preadaptation in convergent evolutionary responses in insects, but our understanding of the initial genomic "starting conditions" in these systems is limited by the difficulty of reconstructing the initial mutational events that cause adaptive traits [22, 23]. Understanding how genetic "hot spots" constrain functional pathways available for adaptive responses can sharpen our power to predict which genes or gene families are targets of selection [24], and characterization of the genomic architecture of a rapidly evolving convergent phenotype such as flatwing will inform the core mechanisms that generate and maintain biological diversity. The behavioral and morphological manifestations of the flatwing phenotype on Kauai and Oahu, and their genetic and genomic bases, are most parsimoniously explained by a scenario in which the wing-silencing mutation arose twice independently on the X chromosome and then increased in frequency under similar natural selection regimes. The swiftness with which this process has occurred in *T. oceanicus* confirms observations from experimental evolution studies [25] and studies that have inferred historical convergence on the basis of both genetic and structural homology [26].

Experimental Procedures

Population Sampling

We used laboratory populations of crickets from Kauai and Oahu that were originally established in 2003 and 1991, respectively, and supplemented approximately once per year thereafter with the offspring of wild-caught females. Flatwings from Hawaii were not considered in this study because the low proportion of flatwings present in the population prohibits adequate sampling and the study was initiated before the appearance of flatwings on Hawaii. Both the Kauai and Oahu lab populations contained a mix of flatwing and normal males. Crickets were reared in a common-garden environment at 25°C on a 12 hr light:12 hr dark cycle. They were provided Fluker's Cricket Chow, Purina Rabbit Chow, and water ad libitum.

Morphometric Analysis

Using fw^K (n = 110), fw^O (n = 88), normal Kauai (N^K, n = 64), and normal Oahu (N^O, n = 53) males reared in a common-garden lab environment, we placed 16 landmarks on individual wing images using the program tpsDIG v2.16 [27]. The Integrated Morphometrics Package [28, 29] was then used to calculate Procrustes distances and quantify differences among populations and wing morphs. We first performed PCAs and MANOVA to test for statistical differences between groups in the absence of a priori information about group identity. We then performed canonical variates analysis to quantify and visualize wing shape differences between groups [30]. Two independent morphometric validation studies were performed using wings from common-garden lab-reared crickets used in the subsequent RAD-seq bulk segregant analysis and a sample of Kauai and Oahu flatwing males from the wild. Additional details are provided in the Morphometric Analysis section of the Supplemental Experimental Procedures.

Mode of Inheritance

The mode of inheritance in the Oahu population was established by performance of a series of standard Mendelian crosses [13]. Grylline crickets have an XX/XO (female/male) sex-determination system, and the mutation is sex limited because it feminizes male wings. Using a *T. oceanicus* population that contained no flatwing males, we performed replicated parental crosses, F_1 crosses, and bidirectional backcrosses. We tested whether the frequency of flatwing versus normal progeny for each cross differed from expectations under three modes of inheritance using goodness-of-fit G tests, and we used heterogeneity G tests to verify that F_2 and BC1 progeny ratios did not differ among families [31]. Details of the crossing design and analyses are provided in the Mode of Inheritance section of the Supplemental Experimental Procedures.

Bulk Segregant Analysis

Genomic DNA from cricket heads was individually extracted from lab-reared fw^K, N^K, fw^O, and N^O males (n = 100 individuals for each pool). RAD library preparation was carried out following Baird et al. [14], with some modifications. Demultiplexing of RAD reads and clustering of the loci within and between populations were performed using STACKS v0.9999. Paired-end Read2 was assembled using IDBA_UD v1.0.9, and Read1 consensus and Read2 contigs were merged using EMBOSS merger v6.5.7. Merged sequences were used as a reference, and the reads were mapped back to this reference as pairs. To identify candidate SNPs, we required at least a depth of coverage of ten in each sample, a different major allele detected in at least one mutant/normal pair, and the variable position to be located in the Read1 or in a merged Read1/Read2 consensus. For the identification of flatwing-associated candidate markers, we used a major allele frequency of at least 0.95. To exclude the possibility of sample mislabeling, we performed Sanger sequencing validations for ten SNP markers by selecting discriminating markers for each one of the four different samples. Details of DNA extraction, RAD-seq methodology, and validations can be found in the RAD-seq and Bulk Segregant Analysis section of the Supplemental Experimental Procedures.

Accession Numbers

RAD tag reads have been deposited in the European Nucleotide Archive (accession number ERP005492).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, two figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.04.053.

Acknowledgments

We are grateful to David Forbes for helping to maintain crickets in the lab and Catherine Eland for RAD library preparation. Kirsten Klappert generously helped to estimate genome size of the crickets, and John Rotenberry provided helpful feedback on the manuscript. This research was funded by Natural Environment Research Council grants (NE/G014906/1 to N.W.B. and NE/I027800/1 to N.W.B. and M.G.R.) and by National Science Foundation grants to M.Z. Sequencing and bioinformatics work was supported by the NERC Biomolecular Analysis Facility at the University of Edinburgh (NBAF655).

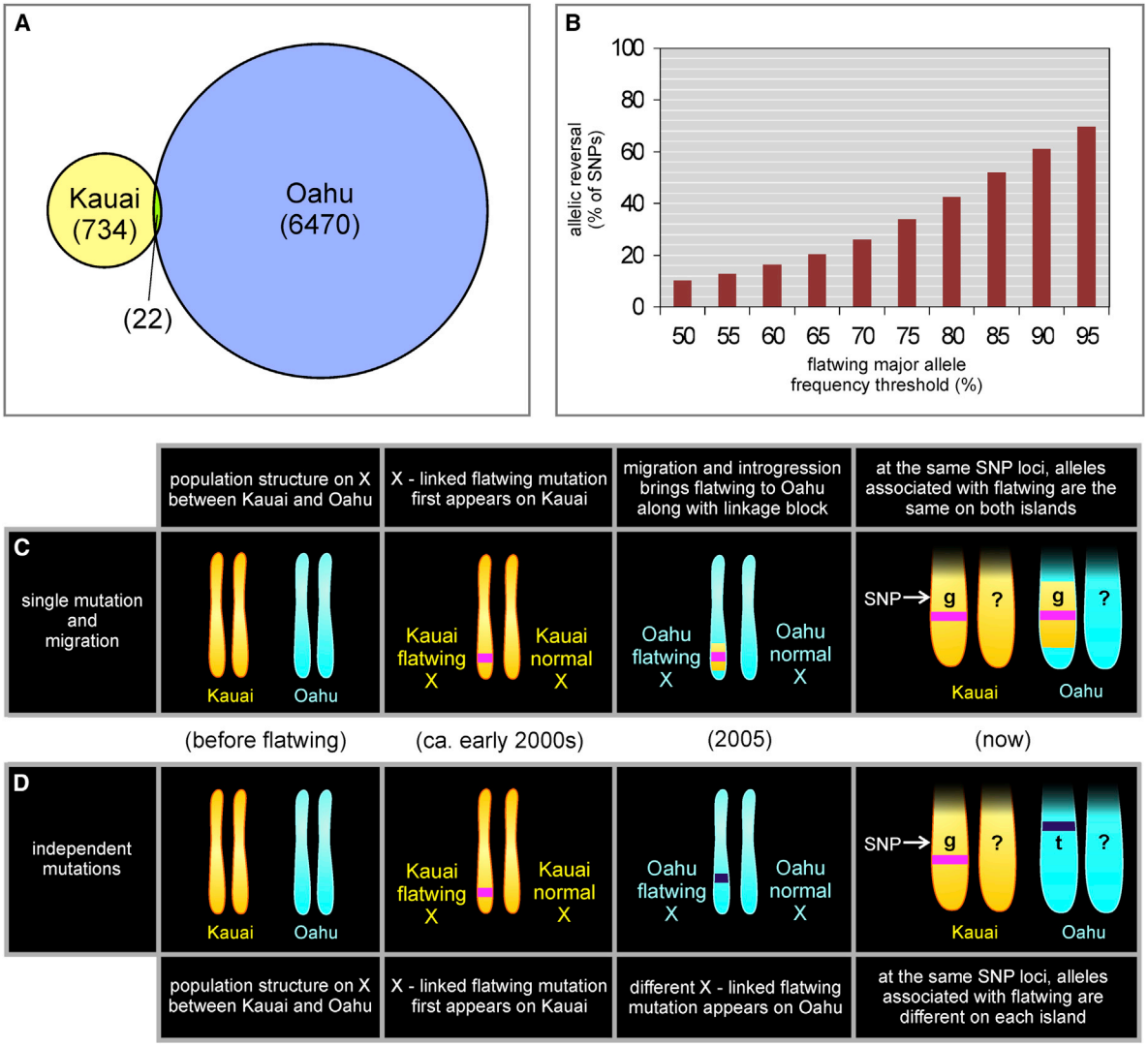

Figure 3. Genomic Analyses of RAD-Derived SNPs Associated with flatwing Morphology and Evolutionary Genetic Hypotheses for the Occurrence of Distinct flatwing Polymorphisms on Kauai versus Oahu

(A) Venn diagram of the associated markers in each population. The number of associated SNPs (in parentheses) was an order of magnitude smaller in Kauai, and the two populations shared 22 associated SNPs in common (2.9% of all Kauai markers, 0.3% of all Oahu markers).

(B) The total proportion of SNPs showing allelic reversal in Kauai versus Oahu crickets in the BSA. As the threshold for calling an associated SNP is increased, indicating greater confidence in a SNP being in linkage disequilibrium with the causal mutation(s), the proportion of SNPs showing allelic reversal between the two populations increases (red bars).

(C and D) Flatwing segregates as a single-locus, X-linked trait on both islands but causes different degrees of wing alterations on each. The approximate timeline of the phenotype's evolution is indicated in the center. (C) shows hypothesis 1: the causal X-linked sequence mutation arose on Kauai and introgressed into the genomic background of Oahu after a migration event. SNP alleles associated with flatwing are expected to be identical on either island because of the Kauai background still physically linked to the causal flatwing mutation that recombination has not yet eliminated. (D) shows hypothesis 2: the causal sequence mutation underlying flatwing first arose on a Kauai X chromosome and then independently arose on an Oahu X chromosome. If flatwing appeared on X chromosomes carrying different SNP alleles at loci in linkage disequilibrium with the mutation, then alleles at SNPs associated with flatwing are expected to differ between the two populations. Our data are consistent with the latter hypothesis.

See also Figure S2 and Tables S2 and S4.

Received: February 28, 2014
Revised: April 23, 2014
Accepted: April 25, 2014
Published: May 29, 2014

References

1. Hall, B.K. (2003). Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. Biol. Rev. Camb. Philos. Soc. *78*, 409–433.
2. Mueller, R.L., Macey, J.R., Jaekel, M., Wake, D.B., and Boore, J.L. (2004). Morphological homoplasy, life history evolution, and historical biogeography of plethodontid salamanders inferred from complete mitochondrial genomes. Proc. Natl. Acad. Sci. USA *101*, 13820–13825.
3. Zuk, M., Rotenberry, J.T., and Tinghitella, R.M. (2006). Silent night: adaptive disappearance of a sexual signal in a parasitized population of field crickets. Biol. Lett. *2*, 521–524.
4. Faria, R., Renaut, S., Galindo, J., Pinho, C., Melo-Ferreira, J., Melo, M., Jones, F., Salzburger, W., Schluter, D., and Butlin, R. (2014). Advances in ecological speciation: an integrative approach. Mol. Ecol. *23*, 513–521.

5. Arendt, J., and Reznick, D. (2008). Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends Ecol. Evol. *23*, 26–32.
6. Elmer, K.R., and Meyer, A. (2011). Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends Ecol. Evol. *26*, 298–306.
7. Parker, J., Tsagkogeorga, G., Cotton, J.A., Liu, Y., Provero, P., Stupka, E., and Rossiter, S.J. (2013). Genome-wide signatures of convergent evolution in echolocating mammals. Nature *502*, 228–231.
8. Wake, D.B. (1991). Homoplasy: the result of natural selection, or evidence of design limitations? Am. Nat. *138*, 543–567.
9. Dugatkin, L.A. (2008). Principles of Animal Behavior, Second Edition (New York: W.W. Norton and Company).
10. Heliconius Genome Consortium (2012). Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature *487*, 94–98.
11. Kim, M., Cui, M.-L., Cubas, P., Gillies, A., Lee, K., Chapman, M.A., Abbott, R.J., and Coen, E. (2008). Regulatory genes control a key morphological and ecological trait transferred between species. Science *322*, 1116–1119.
12. Payseur, B.A., Krenz, J.G., and Nachman, M.W. (2004). Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. Evolution *58*, 2064–2078.
13. Tinghitella, R.M. (2008). Rapid evolutionary change in a sexual signal: genetic control of the mutation '*flatwing*' that renders male field crickets (*Teleogryllus oceanicus*) mute. Heredity (Edinb) *100*, 261–267.
14. Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., and Johnson, E.A. (2008). Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE *3*, e3376.
15. Tinghitella, R.M., Zuk, M., Beveridge, M., and Simmons, L.W. (2011). Island hopping introduces Polynesian field crickets to novel environments, genetic bottlenecks and rapid evolution. J. Evol. Biol. *24*, 1199–1211.
16. Béthoux, O. (2010). Alteration of sex-related developmental modules: a case of "feminized" male wing morphology in *Creobroter gemmatus* (Mantodea: Hymenopodidae). Eur. J. Entomol. *107*, 133–135.
17. Whiting, M.F., Bradler, S., and Maxwell, T. (2003). Loss and recovery of wings in stick insects. Nature *421*, 264–267.
18. Roff, D.A. (1986). The evolution of wing dimorphism in insects. Evolution *40*, 1009–1020.
19. Ritchie, M.G., Butlin, R.K., and Hewitt, G.M. (1987). Causation, fitness effects and morphology of macropterism in *Chorthippus parallelus* (Orthoptera: Acrididae). Ecol. Entomol. *12*, 209–218.
20. Kowalko, J.E., Rohner, N., Rompani, S.B., Peterson, B.K., Linden, T.A., Yoshizawa, M., Kay, E.H., Weber, J., Hoekstra, H.E., Jeffery, W.R., et al. (2013). Loss of schooling behavior in cavefish through sight-dependent and sight-independent mechanisms. Curr. Biol. *23*, 1874–1883.
21. Johnson, J.B. (2001). Adaptive life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator-induced plasticity. Evolution *55*, 1486–1491.
22. Hartley, C.J., Newcomb, R.D., Russell, R.J., Yong, C.G., Stevens, J.R., Yeates, D.K., La Salle, J., and Oakeshott, J.G. (2006). Amplification of DNA from preserved specimens shows blowflies were preadapted for the rapid evolution of insecticide resistance. Proc. Natl. Acad. Sci. USA *103*, 8757–8762.
23. Stern, D.L. (2013). The genetic causes of convergent evolution. Nat. Rev. Genet. *14*, 751–764.
24. Stern, D.L., and Orgogozo, V. (2009). Is genetic evolution predictable? Science *323*, 746–751.
25. Chan, Y.F., Jones, F.C., McConnell, E., Bryk, J., Bünger, L., and Tautz, D. (2012). Parallel selection mapping using artificially selected mice reveals body weight control loci. Curr. Biol. *22*, 794–800.
26. Tishkoff, S.A., Reed, F.A., Ranciaro, A., Voight, B.F., Babbitt, C.C., Silverman, J.S., Powell, K., Mortensen, H.M., Hirbo, J.B., Osman, M., et al. (2007). Convergent adaptation of human lactase persistence in Africa and Europe. Nat. Genet. *39*, 31–40.
27. Rohlf, F.J. (2012). tpsDIG: Digitize Landmarks and Outlines, Version 2.16 (Stony Brook: Department of Ecology and Evolution, State University of New York at Stony Brook).
28. Zelditch, M.L. (2012). Morphometrics Software: IMP6-Integrated Morphometrics Package. http://www.canisius.edu/~sheets/morphsoft.html.
29. Rohlf, F.J., and Slice, D. (1990). Extensions of the Procrustes method for the optimal superimposition of landmarks. Syst. Zool. *39*, 40–59.
30. Ashburner, J., Hutton, C., Frackowiak, R., Johnsrude, I., Price, C., and Friston, K. (1998). Identifying global anatomical differences: deformation-based morphometry. Hum. Brain Mapp. *6*, 348–357.
31. Sokal, R.R., and Rohlf, F.J. (1981). Biometry, Second Edition (New York: W.H. Freeman and Company).